Antimicrobial Activity of Date Palm (Phoenix dactylifera) Pits extracts and its role in reducing side effect of Methyl prednisolone on the some Neurotransmitter content in the Brain, Hormone Testosterone in adulthood

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ABSTRACT

The present work was carried out to study the impact of date palm pits as antibacterial activities on two species of pathogenic bacteria (*klebsiella pneumonia* and *Escherichia coli*) and its role in reducing side effect of methylprednisolone on the some neurotransmitter content in the brain, hormone testosterone and testclture of male albino rats .Date palm pits is most effective in inhibiting growth of bacteria as compared with antibiotics due to differences in resistance of bacteria to anti-tested materials due to change in membrane permeability of cells, thereby hindering the entry of enzymes or excreted by the change in the chemical composition of the constituent chemical**.** The results showed that the daily oral administration of pits of date palm caused the maximal increase in NE, DA& GABA content was found in the brain stem after 2 weeks. The daily oral administration of methylprednisolone caused decrease in NE,DA& GABA content was found in the brain stem after 2 weeks. Moreover, the daily oral administration of pits of date palm and methylprednisolone caused increase in NE content was found in the brain stem after 2 weeks. The daily oral administration of pits of date palm and methylprednisolone caused a significant increase in testosterone level in serum blood of male albino rat. From the present results, it is clear that Effect of chronic oral administration of methylprednisolone and pits of data palm on testicular of male albino rat caused recovery effect, notice high sperm in some tubules and tubular partial late spermatogenic arrest (spermatide level) is only seen in 10-20% of tubules.

The appropriate recommendations in this study to use nuclei dates Antimicrobial on *klebsiella pneumonia* and *Escherichia coli* than the activity of standard antibiotics and the results concluded that using intended dates date palm pits as a preventive measure to reduce the side effects resulting from the use of a drug methylprednisolone on the some neurotransmitter content in the Brain. Hormone Testosterone and testclture in male albino rats.

Key word: Pits of Date Palm (Phoenix dactylifera) , Antimicrobial, Methyl prednisolone, *klebsiella pneumonia* , *Escherichia coli*, Neurotransmitter content.

INTRODUCTION

The date palm (*Phornix dactylifera L*.) is considered the most important source of food for both human in arid and semiarid (Besbes *et al.,* 2004). Dates contain a high percentage of sugars reaching 88% in some varieties (Al Shahib and Marshall,2003). Dates are also rich in mineral salts and vitamins (Booij *et al.*,1992). For the date pit, the percentage of non-reducing sugars is 3.82% and in glucose and fructose is 1.68 and 1.53, respectively (Fayadh and Al-Showiman,1990). In local medicinal practices, dates are considered a tonic. Some consider it to be an aphrodisiac. The flower of the plant is used as a purgative (Zohget *et al.*,2000). Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats (El-Mougy *et al*., 1991). Date palm pollen(DPP) cure

mal infertility by improving the quality of sperm parameters (Bahmanpour *et al*., 2006). Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of testosterone (Ali *et al.*, 1999).

The pits of *Phoenix dactylifera* contains different chemical compounds such as saturated and unsaturated fatty acids, Zinc (Zn), Cadmium (Cd), Calcium (Ca), and potassium (K). Saturated fatty acids include stearic and palmitic acid and unsaturated fatty Acids contain linoleic and oleic acids which could inhibit 5 -α reeducates enzyme (Shariati *et al*., 2008). Also, dates contain at least six vitamins including a small amount of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A (Al-Shahib and Marshall ,1993). Studies indicate that the aqueous extracts of dates have potent antioxidant activity (Mansouri *et al*.,2005). The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins (Gu *et al*.,2003 and Al-Farsi *et al*.,2005).

In recent years, it has been suggested that estrogen, may be involved in the regulating the renewal of spermatogonial stem cells (Miura *et al*., 2003) and male reproductive tissues with estrogen receptors (Amin *et al*., 1969). Investigations have revealed that palm kernels and date pollen grains extracts contain estrogenic materials as gonad-stimulating compounds that improve male infertility. Reports have also pointed that isolation of micro elements from DPP has estrogen, sterols, and other agents that may influence male fertility.(,Bennet *et al*., 1966 ; Mahran *et al*., 1976 and Bajpayee *et al*., 1997). With regard to these components, snack foods have been supplemented with date pollen to improve male infertility (Abde-El-Mageed *et al*., 1987).

The present work was carried out to study the impact of date palm pits as antibacterial activities on two species of pathogenic bacteria (*klebsiella pneumonia* and *Escherichia coli*) and its role in reducing side effect of methylprednisolone on the some neurotransmitter content in the brain, level of hormone testosterone and testclture in male albino rats.

MATERIALS AND METHODS

1. Materials

1.1*:* Date palm *(Phoenix Dactylifera* L.) Pits

It belongs to Kingdom: Plantae, Division: Magnoliophyta, Class: Liliopsida, Order: Arecales , Family: Arecaceae, Genus: Phoenix, Species: Phoenix dactylifera and the binomial mane: *Phoenix dactylifera* Linn. Date fruits were obtained from the Al-Gaseem Date Factory in the central region of the Kingdom of Saudi Arabia.

1.2 Drug

Methylprednisolone at a dose of 20 mg / kg through the tube infectious oral once daily (Park, 1998**).**

1.3: Test Bacterial

Klebsiella pneumonia and *Escherichia coli* from Laboratory of Jeddah Hospital in the kingdom of Saudi Arabia. It was cultured on Mueller Hinton media (Oxioid CM 41) at 37°C .

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin ،and intestines (Ryan and Ray , 2004). It is clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae; *Klebsiella* was named after the German bacteriologist Edwin Klebs (1834–1913).

Escherichia coli is a Gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). It is now classified as part of the Enterobacteriaceae family of gamma-proteobacteria (Thompson, 2007). Lee, *et al*.,(2009) found that pathogenic *E. coli* are found in meat in Korea, and could act as a transmission vehicle for human infection as suggested by the occurrence and classification of pathogenic *E. coli* in retail meats.

Furthermore, the data from their study could be used in the risk assessment of foodborne illnesses linked to meat consumption.

1.4: Animals

Adult Male albino rats, and weighing (100 to 120 g), were obtained from the Experimental Animal House Center, , King abdualaziz University, Jeddah, Saudi Arabia. All animals were given food (rat chow or date extract) and water ad libitum, and were maintained at a relative humidity of 65% to 86%, a temperature of 23°C to 25°C, and in a schedule of 12 hours of light and 12 hours of dark. Rats were weighed at the beginning and end of the study. Procedures involving animals and their care were conducted in conformity with international laws and policies.

1.5: Standard antibiotic disc :

Amikacin ,Gentamicin , Imipene,Cefotaxime,Cefe-pime, Aztreonam, Piperacillin , Amoxicillin – Clavulanate, Tazobactam, Colistin ,Nitrofurantion ,Ciprofloxacin ,and Norfloxacin Mast Diagnostic Amiens , France.

2. The Methods

2.1- Preparation of *Phoenix Dactylifera* (Date palm) Pits:

The dried pits were ground into a fine powder and immersed in cold distilled water (1:3 ratio, weight to volume) for 48 hours at a temperature of 4°C. The water extract was prepared freshly and given to the animals ad libitum in place of rat chow. (Al-Qarawi *et al*., 2004).

2.2- Antibacterial activities :

The agar disc diffusion method was employed for the determination of antibacterial activities of the water extract of *Phoenix Dactylifera* Pits powder (Hasenekoğlu ,1990). Suspension of the tested microorganisms (10^6 CFU/µl) was spread on Mueller Hinton Agar (Oxioid) for bacteria, filter paper discs (6 mm in diameter) were soaked with 20 µl of the stock solutions and placed on the inoculated plates. After keeping at 2 °C for 2 h, they were incubated at 37 °C for 24 h . The diameter of the inhibition zones were measured in millimeters. Some known antibiotics Amikacin ,Gentamicin , Imipene, Meropenem, Cephalothin, Cefuroxime, Ceftazidime. Cefotaxime, Cefe-pime, Aztreonam, Ampicillin ,Piperacillin, Amoxicillin-Clavulanate, Piperacillin-Tazobactam, Colistin, Nitrofurantion, Ciprofloxacin, Norfloxacin and Tetracyclin were evaluated for their antibacterial activities and their results compared with *Phoenix Dactylifera* Pits extract (Baker and Breach ,1980).

2-3. Animal treatment:

The animals were divided into four groups (n=4) of equal number, three experimental and control groups. The control group only received an equal volume of distilled water. The control group (1) that was orally and daily administered the equivalent amount of the vehicle (distilled water) for the same period. The second group was orally and daily administered methylprednisolone at a dose of 20 mg / kg and third group was orally and daily administered pits of date palm (20 mg /kg b.w.t). Later, third group was orally and daily administered 4 ml of pits of data extract (20 mg /kg b.w.t). For 15 consecutive days and treated with methylprednisolone (20mg/kg) with pits of data extract. the end of the experimental periods (2 weeks), rats were scarified under diethyl ether anesthesia at fasting state.

Duration the treatments four rats of each group were decapitated each week till the end of the 2-week duration times. The rats were killed by sudden decapitation at the designed times. The brain was rapidly and carefully excised, and was then dissected according to the method of Glowinski and Lversen (1966) into the following regions; cerebellum, striatum cerebral cortex, hypothalamus, brain stem and hippocampus. brain content were wiped dry with filter paper, weighed , wrapped in plastic films and then in aluminum foil and were quickly frozen in dry ice, pending analysis. NE and DA were extracted and estimated in the brain tissues according to

the method of Chang (1964) modified by Ciarlone (1978). GABA were extracted and estimated in the brain tissues according to the method of(Sutton & Simmonds1973) .

2-4. Blood Sampling

The portion of blood samples were collected and allowed to coagulate at room temperature; EDTA (ethylene diamine tetracetic acid) was added to the other portion of blood and centrifuged at 3000 r.p.m. for 30 minutes. The clear, non-haemolysed supernatant serum and plasma were quickly removed divided into four portions for each individual, and stored at -20°C for subsequent analysis. For the measurement of testosterone using immunoassay technique and Spectra. Testosterone kits were used according to their manufacturer's instruction (Orion Diagnostica; Finland and DRG Instruments GmbH; Germany).

2-5. Histological studies

After sacrifice of animals, part of the testclture from each animal from treated and control was removed and immersed in 10% buffered formalin solution. testclture was kept in separate numbered small glass bottles. Testclture was then embedded in paraffin, and sectioned. Four sections (5 microns in thickness) were taken from each testcluture, each section being at a distance of at least 500u from the proceeding one Sections were stained with haematoxylin and eosin (Harris,1900). Determination the scoring system for tubular affection *(mean no. of dysfunctioning tubules per 5 fields x100)*. 0-1 represented to All tubules show active spermatogenesis 1-2 represented to a portion of tubules show arrest or hypospermatogenesis 2-3 represented to All tubules show arrest or hypospermatogenesis, but germ cells are intact. 3 represented to All tubules show arrest or hypospermatogenesis, but germ cells are partially or completely replaced.

2-6. Statistical Analysis

Values reported are means ± SE (n = 6). The results were statistically analyzed using the Student's t-test (Hill, 1971) for unpaired data, with P value of less than 0.05 considered significant.

RESULT

Is clear from Table (1) the emergence of the inhibition zone of the growth of pathogenic bacteria *Klebsiella pneumonia* and *Escherichia col*i as a result of various transactions, with extract of pits of data palm. It was found that when the transaction intended to pits of data palm extract report Inhibition zone formed around the filter papers saturated with about 16.351 ± 0.00 and 10.00 ± .032 mm respectively, and similar to the impact of anti-vital Cefotaxime with the impact of intended dates on bacteria *K. pneumonia*. Appearance lesser extent of the inhibition zone of bacteria *E. coli* reaching 4.33 ± 21.0 mm while gives the adversaries Aztreonam and Amikacin results are similar for each of the two genus. Antibiotic Colistin less effect in the inhibition of bacteria tested, reaching 0.833 ± 0.211 and 0.533 ± 0.021 mm for each of the *K. pneumonia* and *E. coli*, respectively.

It is noticed that the difference in the sensitivity of bacteria through the difference in the inhibition zone around disks saturated and date palm pits is most effective in inhibiting growth of bacteria as compared with anti-vital due to differences in resistance of bacteria to anti-tested materials.

The present results in table (2) and Figure (2) showed that the daily oral administration of pits of data palm (20 mg /kg b.w.t) resulted in a significant increase in DA Content Starting from the 1st Week in cerebellum, Cerebral cortex , brain stem and hippocampus and in the same tested areas from the 2nd week till the end of experimental duration. the maximal($p < 0.01$) increase in DA content found after 2 week in cerebellum (+38.69%). Table (3) and figure(3)

shown that the daily oral administration of pits of data palm caused a significant increase in GABA content standing from the 1st week in cerebellum, Striatum, Cerebral cortex and brain stem. from the 2nd week in all tested areas except Hypothalamus and Hippocampus. The maximal ($p< 0.001$) increase in GABA content was found after 2 weeks in brain stem (+324.35%).

As shown table (4) and figure (4) the daily oral administration of pits of data palm (20 mg /kg b.w.t) caused a significant increase in NE content starting from the one week in cerebellum, and from the 2 week in cerebellum and striatum.and the maximal ($p< 0.01$) increase in NE content was found in the cerebellum after 2 weeks (+41.22%).

The present results in table (5) and Figure (5) showed that the daily oral administration of methylprednisolone (20 mg /kg b.w.t) resulted in a significant decrease in DA Content Starting from the 1st Week in all brain area till the end of the experiment duration and in the same tested areas from the 2nd week till the end of experimental duration. the maximal decrease ($p< 0.001$) in DA content found after 2 week in hypothalamus (-78.94%). Also, table (6) and figure (6) showed that the daily oral administration of methylprednisolone caused a significant ($p< 0.001$) decrease in GABA content standing from the 1st Week in all brain area till the end of the experiment duration except Cerebral cortex and in the same tested areas from the 2nd week till the end of experimental duration. the maximal decrease in GABA content found after 2 week in hypothalamus (-77.48%).

The results obtained from table (7) and Figure (7) showed that the daily oral administration of methylprednisolone (20 mg /kg b.w.t) caused a significant decrease in NE content starting from the one week in all brain area till the end of the experiment duration. the maximal decrease($p< 0.001$) in NE content was found in the hippocampus after 2 weeks (-82.05%).

As shown table (8) and Figure (8) the daily oral administration of pits of data palm and methylprednisolone resulted in a significant increase in DA content Starting from the 1st Week in Cerebral cortex and decrease in cerebellum, striatum, brain stem, hypothalamus, and hippocampus. from the 2nd week increase in cerebellum, striatum and cerebral cortex . Decrease in DA content in hypothalamus and hippocampus. the maximal ($p< 0.001$) increase in DA content found after 2 week in cerebral cortex (+312.86%). the maximal ($p< 0.01$) decrease in DA content found after 2 week in striatum (-37.19%). Table (9) and Figure (9) shown that the daily oral administration of pits of data palm and methylprednisolone caused a significant increase in GABA content standing from the 1st week in cerebral cortex. from the 2nd week increase in striatum and cerebral cortex and decrease in all tested areas. The maximal ($p< 0.001$) increase in GABA content was found after 2 weeks in cerebral cortex (+72.84%). The maximal ($p< 0.01$) decrease in GABA content was found after 1 week in hippocampus (-54.68%). As shown table (10) and figure (10) the daily oral administration of pits of data palm and methylprednisolone caused a significant increase in NE content starting from the one week in cerebellum, and from the 2 week in cerebellum and striatum and the maximal ($p< 0.01$) increase in NE content was found in the cerebellum after 2 weeks (+41.22%).

The present results present in Table (10) and figure (11,12&13) shown that the daily oral administration of methylprednisolone caused a significant decrease in testosterone level in serum blood of male albino rat. The maximal ($P< 0.05$) decrease in testosterone level was found in after 2 weeks (-22.89). while the daily oral administration of data palm pits and data palm pits with methylprednisolone caused a significant increase in testosterone level in serum blood of male albino rat. The maximal ($P< 0.001$) increase in testosterone level was found in after 2 weeks (218.46 and 72.37, respectively).

The present results showed that (Plate. 1a&b) histologically, Note small normal seminiferous tubules mostly without lumen, surrounded by fibrous connective tissue layer with high magnification showing dark spermatogonium and pachytene spermatocytes and Sertoli cell.. Also, note, myoid cell Layer surrounded and intertubular space contains leydig cells Plate1c&d

showed that enlarged normal seminiferous tubules populated by spermatocytes and late spermatids surround the tubular Lumen and High power showing Light and dark spermatogonium adjacent to basal Lamina; late spermatids with elongated head directed towards Sertoli cells.

The normal testicular architecture, interstitial cells and tubules show active spermatogenesis with normal central luminal mature sperms. Tubular dysfunction (ie hypospermatogensis & germ cell maturation arrest) are within the normal range. No organic pathological lesions (i.e. absent interstitial fibrosis, congestion, vascular injury or inflammation with no tubular necro-degenerative changes or atrophy).

The results obtained from Plate.2 showed that effect of chronic oral administration of pits of data palm on testicular of male albino rat caused the tubules of testicular showing an increased active spermatogenesis with significant rise of number of mature sperms. No interstitial fibrosis, congestion, vascular injury or inflammation with no tubular necro-degenerative changes or atrophy). As shown plate 2c. Marked increase in spermatogenesis, free of early or late arrest.

The results obtained from Plate 3 showed that effect of chronic oral administration of methylprednisolone on testicular of male albino rat caused marked reduction of spermatogenesis (hypo spermatogenesis) and tubule showing Partial late arrest with marked reduction of mature sperms. As shown plate 3c early arrest with absent mature sperms & germ cell hypoplasia. It noticed in plate 3 d. There are focal areas of disrupted architecture & the tubules show absent spermatogenesis (mostly early arrest). Also, in plate 3e. Foci of interstitial fibrosis, congestion, vascular injury 'endarteritis" & inflammation are also encountered with tubular necro-degenerative changes, as well as atrophy of both germ & interstitial cells.

From the present results, it is clear that Effect of chronic oral administration of methylprednisolone and pits of data palm on testicular of male albino rat caused recovery effect, notice high sperm in some tubules and tubular partial late spermatogenic arrest (spermatide level) is only seen in 10-20% of tubules. There is minimal interstitial fibrosis but no Vascular injury 'end-artritis obliterans', or tubular necro-degenerative changes or atrophy (plate.4)

The results showed that the daily oral administration of pits of date palm caused a significant decrease tubular dysfunction and arrest in testicular of male albino rat. The daily oral administration of methylprednisolone caused increase tubular dysfunction and arrest in testicular. Moreover, the daily oral administration of pits of date palm and methylprednisolone (Recovery) caused reduced tubular dysfunction and arrest testicular (Figure14).

DISCUSSION

Date palm pits is most effective in inhibiting growth of bacteria as compared with antibiotic due to differences in resistance of bacteria to anti-tested materials due to change in membrane permeability of cells, thereby hindering the entry of enzymes or excreted by the change in the chemical composition of the constituent chemical or by changing the nature of some of their components targeted by the anti-abstract (Aba Al-Khail *et al*., 2003). These results agree with Jassim *et al.*,(2007) found that the date pit extracts show a strong ability to inhibit the infectivity of *Pseudomonas* phage ATCC 14209- B1 and completely prevented bacterial lysis. This effect was shown to be due to interference with some aspect of the phage's lytic cycle. The lytic cycle of the phage consists of three major phases (Stewart *et al.*,1998): binding to a suitable host bacterium and injection of its genome; a period of intracellular production of new virions; and then lysis of the cell and release of progeny phage into the environment. This effect was shown to be due to a direct effect of the extract on the phage itself rather than an effect on the host cell**.** This finding is supporting by Mansouri *et al.*,(2005) indicated that the aqueous extracts of dates have potent antioxidant activity. The antioxidant activity is attributed to the wide range of

phenolic compounds in dates including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins (Gu *et al.*,2003 and Al-Farsi *et al.*,2005).

From the present results, it is clear that the daily oral administration of pits of date palm caused reducing side effect of Methylprednisolone on the some neurotransmitter content in the brain and a significant increase in neurotransmitter contents (NE, DA& GABA) in most of the tested brain areas at different time intervals; cerebellum which is responsible for the voluntary movement; pons + medulla oblongata which is responsible of essential reflexive acts; striatum which is a brain region responsible for motor activity; Cerebral cortex is responsible for sensation including visual, auditory and olfactory as well as motor coordination and association, also it responsible for higher mental function such as thinking, planning, reasoning, memory and consciousness and hippocampus. This is key area concerned with learning (Bloom, 2001). brain stem is responsible for integration of coordination of essential reflexive acts such as swallowing, vomiting and respiration (Bloom., 2001).

This is in agreement with the previous studies which suggested that the methanolic extract of *P. dactylifera* possesses significant anxiolytic, analgesic, nootropic and antipsychotic activities, which may be attributed to various mechanisms such as decreased serotonergic and dopaminergic transmission and increased cholinergic transmission. These findings scientifically validated the traditional claim and suggested its valuable role in the treatment of various CNS disorders (Vyawahare *et al*., 2009). Various parts of *P. dactylifera* are widely used in traditional medicine for the treatment of various disorders, which include memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders, etc.(Nadkarni,1976). Date fruit extracts have been reported to possess antiulcer, anticancer, antidiarrheal, hepatoprotective, antimutagenic, antioxidant, aphrodiasiac, antiinflamatory, antimicrobial, antigenotoxic, antihyperlipidemic and nephroprotective activities (Vayalil,*et al*., 2002, Doha *et al*.,2004, Al-Qarawi *et al*., 2005, Ishurda *et al*., 2005, Allaith *et al*.,2005; Bahmanpour *et al*.,2006; Jassim, and Naji., 2007, Abdulla and al Taher.,2008 and Mohamed *et al*.,2008). Additionally, the PD extract did not demonstrate any effect on the muscle coordination, as indicated by the findings with respect to the retard model, suggesting that the inhibitory effect of the extract might be elicited via central mechanisms, not by peripheral neuromuscular blockade, and also ruled out the possibility of neurotoxicity (Dunham and Miya, 1967 and. Amos *et al.*, 2001). Abdullah *et al*., (2004) have been suggested that the reduction of CCl4-induced elevated plasma activities of AST, ALT, ALP, and bilirubin level in animals pre- and post-treated with the aqueous extracts of date flesh or pits shows their ability to restore the normal functional status of the poisoned liver, and also to protect against subsequent CCl4 hepatotoxicity.

The daily oral administration of pits of date palm and methylprednisolone caused a significant increase in testosterone level in serum blood of male albino rat. This result agree with Kostyuk ,(2004) indicated that Date palm pollen suspension increases the plasma levels of estradiol and testosterone and these hormones are found at high concentrations in rat testis and seminal fluids. Also, Zargari,(1999) found that date extracts increase sperm count in guinea pigs and increase the concentration of testosterone, follicle stimulating hormone, and Latinizing hormone in rats. Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of testosterone (Nayernia., 2004).

From the present results, it is clear that Effect of chronic oral administration of methylprednisolone and pits of data palm on testicular of male albino rat caused recovery effect, notice high sperm in some tubules and tubular partial late spermatogenic arrest (spermatide level) is only seen in 10-20% of tubules. There is minimal interstitial fibrosis but no vascular injury 'end-arthritis obliterans', or tubular necro-degenerative changes or atrophy. This finding is supporting by Bahmanpour *et al*., (2006) found that The comparative evaluation between control and experimental groups revealed that consumption of DPP suspensions improved the sperm count, motility, morphology, and DNA quality with a concomitant increase

in the weights of testis and epididymis. It did not significantly affect the weight of the prostate and the seminal vesicle or the histology of the reproductive-content. Zargari *et al*., (1999) have revealed that palm kernels and date pollen grains extracts contain estrogenic materials as gonad-stimulating compounds that improve male infertility. Our data showed that using pits of data palm increases the plasma levels of testosterone. This hormone are found at high concentrations in testis and seminal fluids of rat (Kostyuk *et al*., 2004) . Mahran *et al*., (1976) indicated that date palm contain estradiol and flavonoid components that have positive effects on the sperm quality.

As a conclusion, the appropriate recommendations in this study to use nuclei dates Antimicrobial on *klebsiella pneumonia* and *Escherichia coli* than the activity of standard antibiotics and the results concluded that using intended dates date palm pits as a preventive measure to reduce the side effects resulting from the use of a drug methylprednisolone on the some neurotransmitter content in the Brain., Hormone testosterone in male albino rats. From the present results, it is clear that Effect of chronic oral administration of methylprednisolone and pits of data palm on testicular of male albino rat caused recovery effect, notice high sperm in some tubules. These results confirmed that pits of date palm had beneficial effects on male reproductive activity and improve sperm quality, enhance fertility in the male adult rat. Therefore, it may be useful to solve infertility problems.

TABLE (1) Diameter of inhibition zone of the Date palm (Phoenix Dactylifera L.) Pit against *Klebsiella pneumonia ssp pneumonia* and *Escherichia coli*

Treatmen	Date palm (Phoenix Dactylifera L.) Pit	Amikacin	Gentamicin	Imipene	Cefotaxime	Cefepime	Aztreonam	Amoxicillin Clavulanate	Colistin	Nitrofurantion	Ciprofloxacin	Norfloxacin
	Inhibition zoon(mm)											
Klebsiella pneumonia	16.351±0.00	13.333±1.687	6.00±1.265	1. 00±000	16.33±0.211	16.50±0.224	14.667±0.422	8.667±0.422	0.833±0.211	15.667±0.279	1.167±0.279	1.917±0.083
Escherichia coli	10.00±.032	14.00±0.730	7.00 ±0.632	1.00± 0.00	4.33±0.211	15.00±0.305	15.33±0.422	6.00±0.730	0.533±0.021	1.833±0.105	0.63±0.056	1.933±0.042

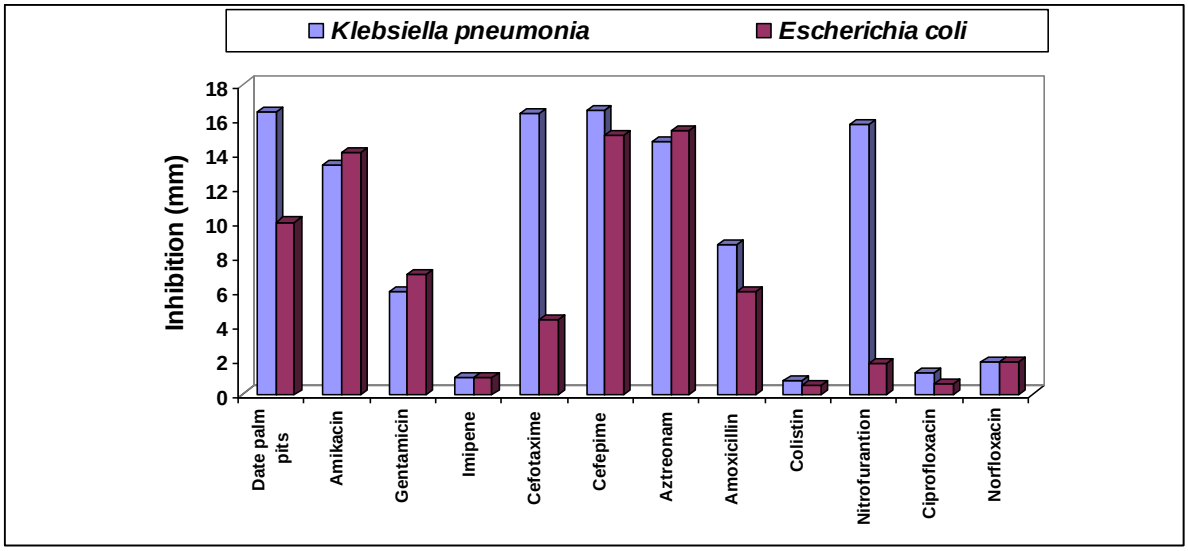

Fig. 1. Diameter of inhibition zone of the Date palm Pits (*Phoenix Dactylifera* L.) Pit againt *Klebsiella pneumonia ssp pneumonia* and *Escherichia coli*.

Table (2) Effect of chronic oral administration of pits of data palm on dopamine (DA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	146.755± 0.818	473.948±0.856	60.488±0.044	734.223±2.111	451.288±0.633	243.147±0.863
	T	200.00± 0.365**	503.000±0.856	69.833±0.307*	743.667±16.936	501.833±0.792	281.000±0.516*
	%	36.28	6.13	15.45	1.29	11.20	15.57
2 weeks	C	145.648±0.914	482.312±3.336	61.240±0.214	739.237±4.314	451.541±1.947	244.597±1.448
	T	202.000±0.966**	503.000±0.856	131.6670.558***	743.667±16.936	506.167±0.477	283.500±0.428
	%	38.69	4.29	115.00	0.60	12.10	15.91

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t'* test
% : Percentage of change from control. *p< 0.05,**p< 0.01 & ***p< 0.001
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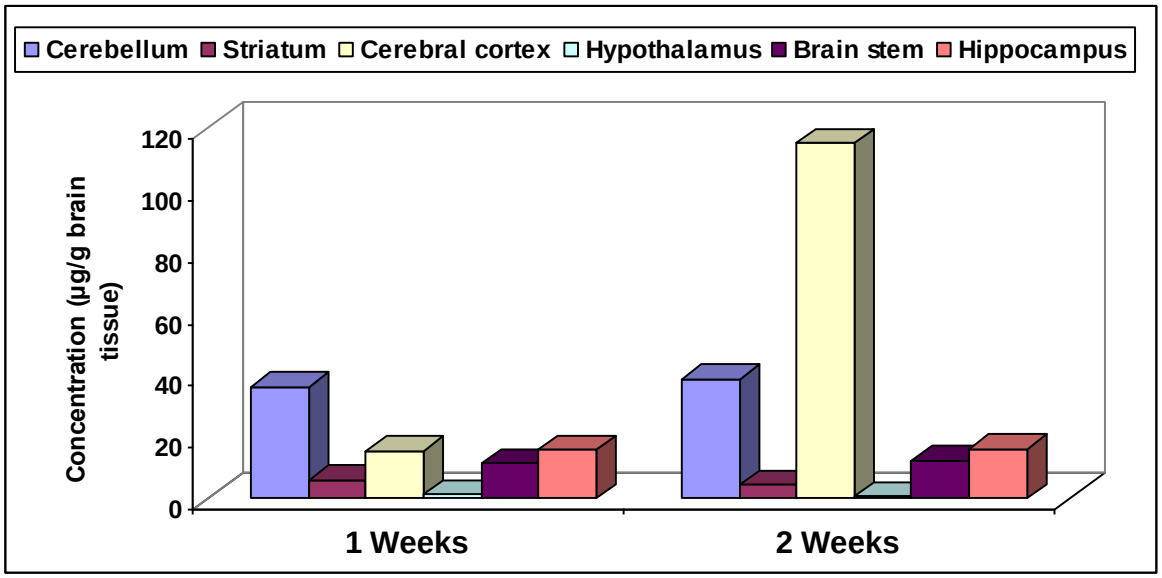


Fig (2) : Effect of chronic oral administration of pits of data palm on dopamine (DA) content represented by the % difference between control and . treated values in the different brain areas of male albino rat

Table (3) Effect of chronic oral administration of pits of data palm on gama-butyric acid (GABA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457±0.799	171.652±0.45	57.247±0.385	432.828±0.319	118.155±0.197	214.78±1.3
	T	221.500±0.428*	204.500±0.34*	64.500±0.342	435.0±0.258	201.3±0.494***	216.35±0.94
	%	15.09	19.14	12.67	0.50	70.40	0.73
2 weeks	C	192.544±0.759	171.662±0.44	57.374±0.463	432.939±0.37	117.868±0.237	214.9±1.27
	T	231.000±0.258*	224.000±0.36*	79.833±0.307**	436.83±0.48	500.167±0.307***	217.6±0.7
	%	19.97	30.49	39.14	0.90	324.35	1.26

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' tes
: Percentage of change from contro *p< 0.05,**p< 0.01 & ***p< 0.001

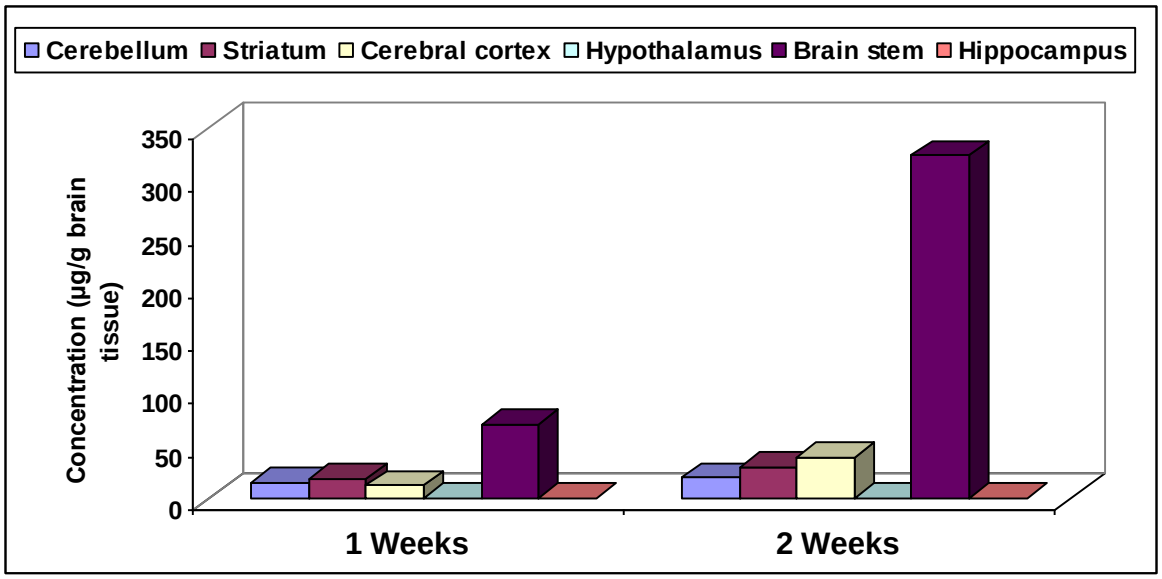


Fig. (3) : Effect of chronic oral administration of pits of data palm on gama-aminobutyric acid (GABA) content represented by the % difference between control and treated values in the different brain areas of male albino rat.

Table (4):Effect of chronic oral administration of pits of data palm on norepinephrine (NE) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	95.382±0.845	511.473±1.803	56.203±0.225	596.997±3.242	390.050±0.831	292.540±1.536
	T	133.500±0.764**	511.673±1.912	56.855±0.276	600.000±0.365	413.667±0.667	293.243±1.263
	%	39.96	0.04	1.16	0.50	6.05	0.24
2 weeks	C	95.358± 0.857	511.118±1.648	54.443±1.898	605.330±9.485	390.490±0.484	292.527±1.531
	T	134.667±0.422**	603.000±0.856	56.688±0.307	600.167±0.307	415.167±0.703	294.433±1.184
	%	41.22 *	17.98 *	4.12	-0.85	6.32	0.65

Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test
: Percentage of change from control *p< 0.05,**p< 0.01 & ***p< 0.001

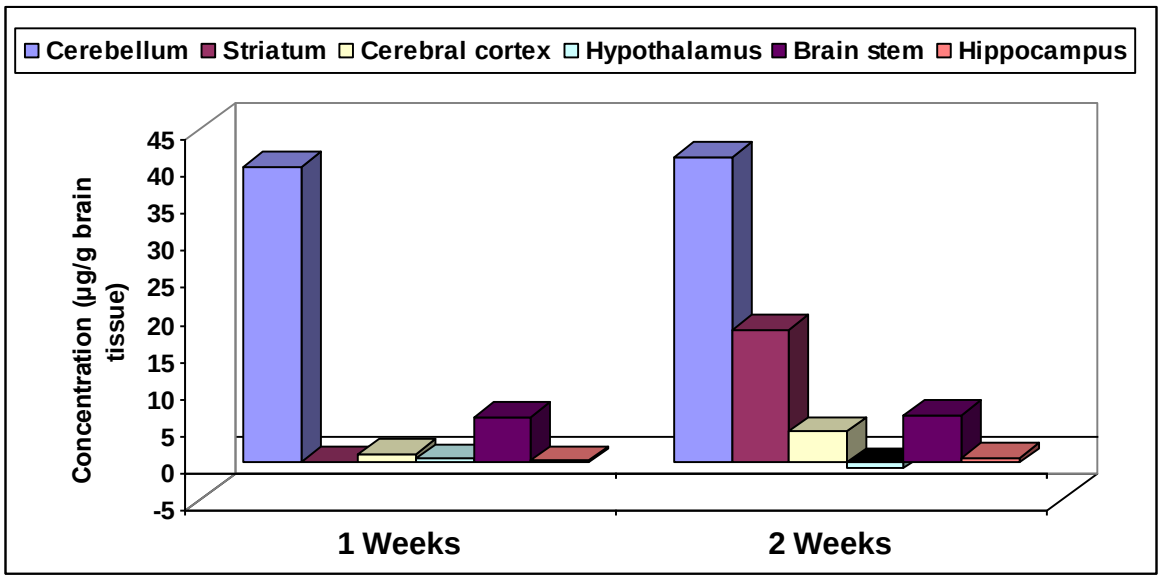


Fig. (4) : Effect of chronic oral administration of pits of data palm on norepinephrine (NE) content represented by the % difference between control and treated values in the different brain areas of male albino rat.

Table (5): Effect of chronic oral administration of methylprednisolone (20 mg/kg b.wt.) on dopamine (DA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	146.755± 0.818	473.948±0.856	60.488±0.044	734.223±2.111	451.288±0.633	243.147±0.863
	T	97.167±1.621*	300.167±0.48*	44.333±0.333*	304.667±1.520**	399.500±0.428	202.167±0.872
	%	-33.79	-36.67	-26.71	-58.50	-11.48	-16.85
2 weeks	C	145.648±0.914	482.312±3.336	61.240±0.214	739.237±4.314	451.541±1.947	244.597±1.448
	T	66.500±1.48**	205.167±1.33**	34.000±0.26**	155.667±1.382***	203.333±1.15**	105.667±1.4**
	%	-54.34	-57.46	-44.48	-78.94	-54.97	-56.80

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test
% : Percentage of change from control *p< 0.05,**p< 0.01 & ***p< 0.001

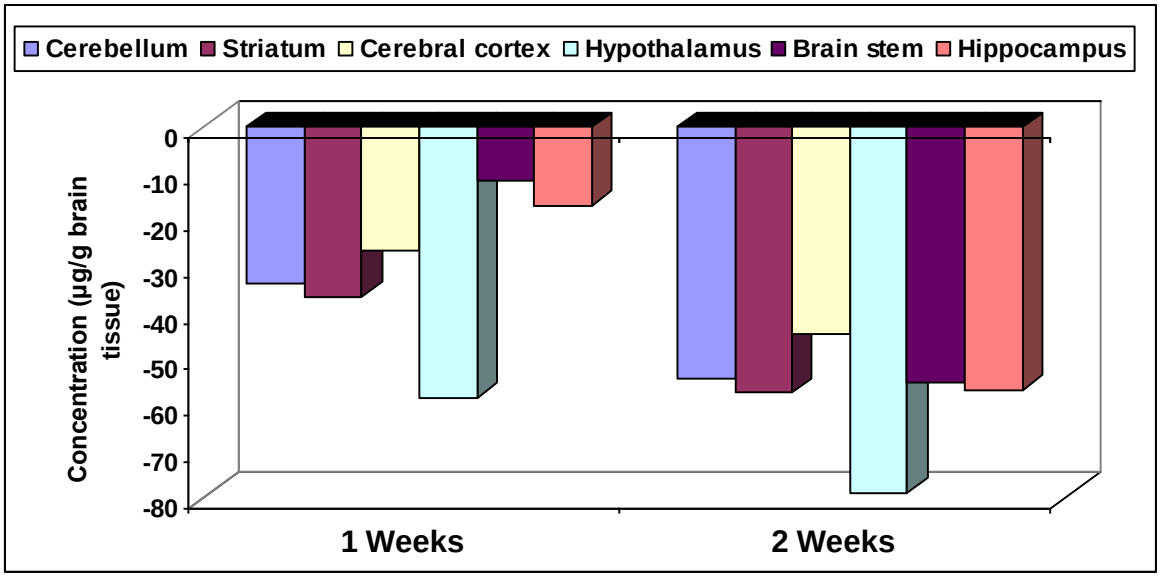
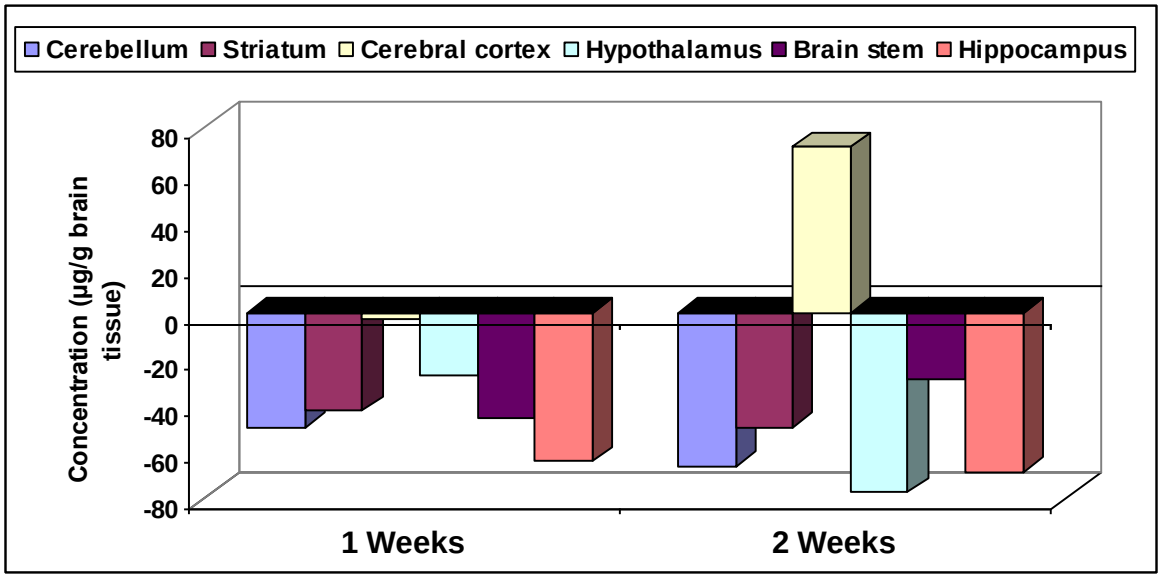


Fig (5) : Effect of chronic oral administration of methylprednisolone (20 mg/kg b.wt.) on dopamine (DA) content represented by the % difference between control and treated values in the different brain areas of male albino rat

Table (6): Effect of chronic oral administration ofmethylprednisolone (20 mg/kg b.wt.) on gama-butyric acid (GABA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457±0.799	171.652±0.450	57.247±0.385	432.828±0.32	118.155±0.197	214.787±1.321
	T	96.833±0.946**	99.000±0.428**	55.667±0.211	315.500±0.43*	64.500±0.764**	77.000±0.577**
	%	-49.69	-42.33	-2.76	-27.11	-45.41	-64.15
2 weeks	C	192.544±0.759	171.662±0.447	57.374±0.463	432.939±0.37	117.868±0.24	214.933±1.269
	T	65.333±0.715***	87.000±0.577**	98.500±0.428***	97.500±0.9***	84.500±0.428*	67.000±0.856***
	%	-66.07	-49.32	71.68	-77.48	-28.31	-68.83

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
% : Percentage of change from control. *p< 0.05,**p< 0.01 & ***p< 0.001



Figure(6): Effect of chronic oral administration of methylprednisolone(20 mg/kg b.wt.)on gama-aminobutyric acid (GABA) content represented by the % difference between control and treated values in the different brain areas of male albino rat .

Table (7): Effect of chronic oral administration of methylprednisolone(20 mg/kg b.wt.) on norepinephrine (NE) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	95.382±0.845	511.473±1.803	56.203±0.225	596.997±3.242	390.050±0.831	292.540±1.536
	T	54.667±0.422***	301.000±0.516**	33.833±0.792*	304.833±0.703**	202.833±0.703**	101.833±0.792***
	%	-42.69	-41.15	-39.80	-48.94	-48.00	-65.19
2 weeks	C	95.358±0.857	511.118±1.648	54.443±1.898	605.330±9.485	390.490±0.484	292.527±1.531
	T	40.500±0.428***	250.500±0.342**	17.667±0.96***	201.167±0.48***	154.333±0.92***	52.500±0.764***
	%	-57.53	-50.99	-67.55	-66.77	-60.48	-82.05

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test.
% : Percentage of change from control. *p< 0.05,**p< 0.01 & ***p< 0.001
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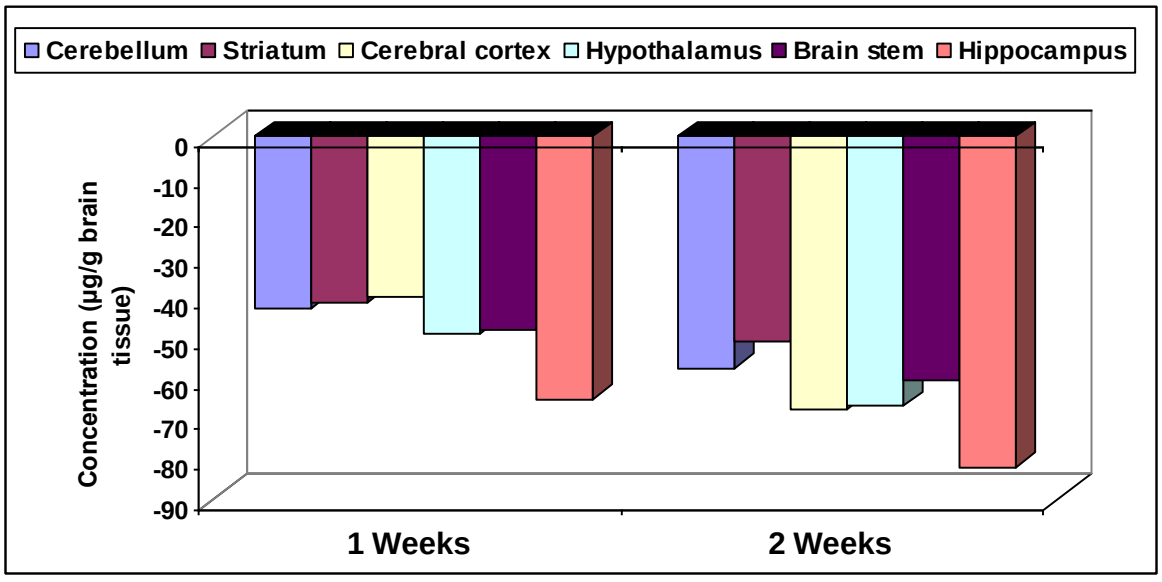

Fig. (7) : Effect of chronic oral administration of methylprednisolone(20 mg/kg b.wt.) on norepinephrine (NE) content represented by the % difference between control and treated values in the different brain areas of male albino rat .

Table (8): Effect of chronic oral administration of pits of data palm and methylprednisolone (20 mg/kg b.wt.) on dopamine (DA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	146.755±0.818	473.948±0.856	60.488±0.044	739.237±4.314	451.288±0.633	243.147±0.863
	T	96.3±1.453***	297.667±0.715***	116.17±0.48***	628.8±0.6*	506.167±0.477	201.667±0.667*
	%	-34.36	-37.19	92.05	-14.93	12.16	-17.06
2 weeks	C	145.648±0.914	482.312±3.336	61.240±0.214	739.237±4.314	451.541±1.947	244.597±1.448
	T	315.5±0.2***	601±0.516*	252.8±0.95***	623±0.58*	491.3±0.8	198.500±0.2*
	%	116.62 *	24.61 *	312.86 *	-15.72 *	8.81	-18.85 *

Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test
: Percentage of change from control *p< 0.05,**p< 0.01 & ***p< 0.001

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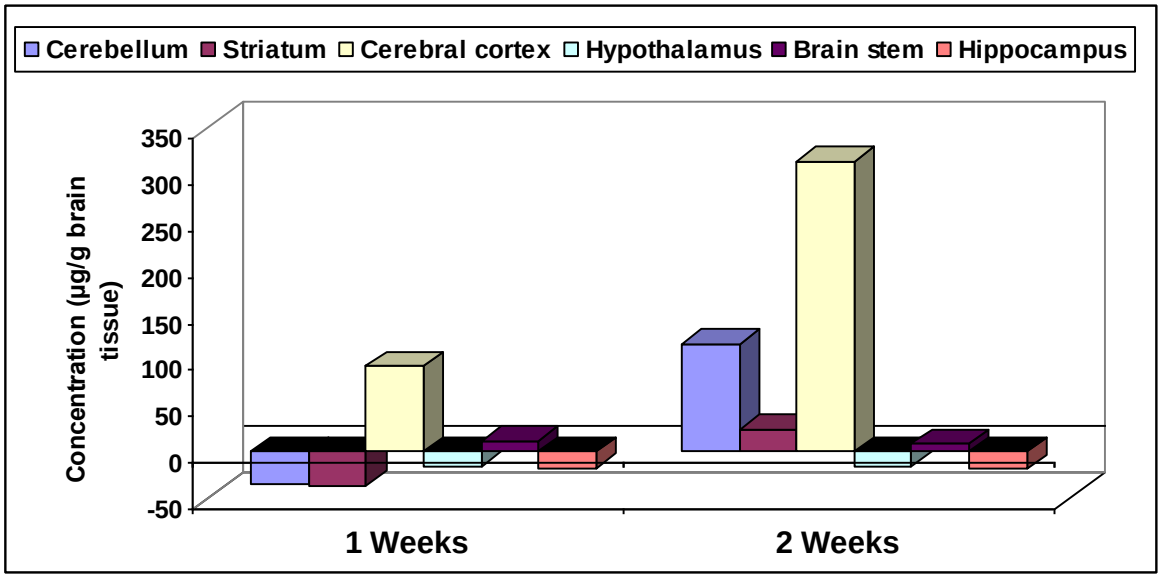

Fig (8) : Effect of chronic oral administration of pits of data palm and methylprednisolone(20 mg/kg b.wt.) on dopamine (DA) content represented by the % difference between control and treated values in the different brain .areas of male albino

Table (9): Effect of chronic oral administration of pits of data palm and methylprednisolone(20 mg/kg b.wt.) on gama-butyric acid (GABA) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	192.457±0.799	171.652±0.450	57.247±0.38	432.828±0.3	118.155±0.2	214.787±1.321
	T	104.000±0.856**	122.667±0.803*	97.500±0.92***	401.000±0.5	99.0±0.4*	97.333±0.843**
	%	-45.96	-28.54 *	70.31 *	-7.35	-16.21 *	-54.68 *
2 weeks	C	192.544±0.759	171.662±0.447	57.374±0.46	432.939±0.4	117.868±0.2	214.933±1.269
	T	102.167±0.98**	206.1670.872*	99.167±0.307***	399.167±1.078	99.167±0.40*	140.000±0.931**
	%	-46.94	20.10 *	72.84 *	-7.80	-15.87 *	-34.86 *

Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test
: Percentage of change from control *p< 0.05,**p< 0.01 & ***p< 0.001

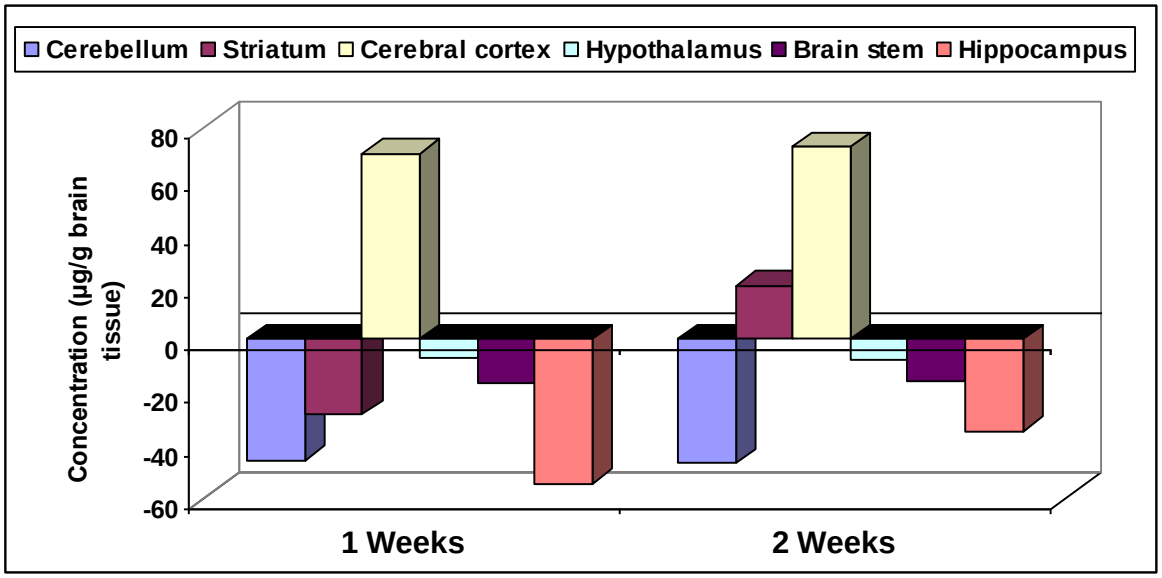

Fig. (9) : Effect of chronic oral administration of pits of data palm and methylprednisolone(20 mg/kg b.wt.)on gama-aminobutyric acid (GABA) content represented by the % difference between control and treated values in the different brain areas of ma

Table (10): Effect of chronic oral administration of pits of data palm and methylprednisolone(20 mg/kg b.wt.)- on norepinephrine (NE) content in the different brain areas of male albino rat.

Time of decapitation		Cerebellum mean ± S.E.	Striatum mean ± S.E.	Cerebral cortex mean ± S.E.	Hypothalamus mean ± S.E.	Brain stem mean ± S.E.	Hippocampus mean ± S.E.
1 week	C	95.382±0.845	511.473±1.803	56.203±0.225	596.997± 3.242	390.050 ± 0.831	292.540±1.536
	T	133.500±0.764	511.673±1.912	56.855±0.276	600.000± 0.365	413.667 ± 0.667	293.243±1.263
	%	39.96 *	0.04	1.16	0.50	6.05	0.24
2 weeks	C	95.358±0.857	511.118±1.648	54.443±1.898	605.330± 9.485	390.490 ± 0.484	292.527±1.531
	T	134.667±0.422	603.000±0.856	56.688±0.307	600.167± 0.307	415.167 ± 0.703	294.433±1.184
	%	41.22 *	17.98 *	4.12	-0.85	6.32	0.65

Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test
: Percentage of change from control *p< 0.05,**p< 0.01 & ***p< 0.001

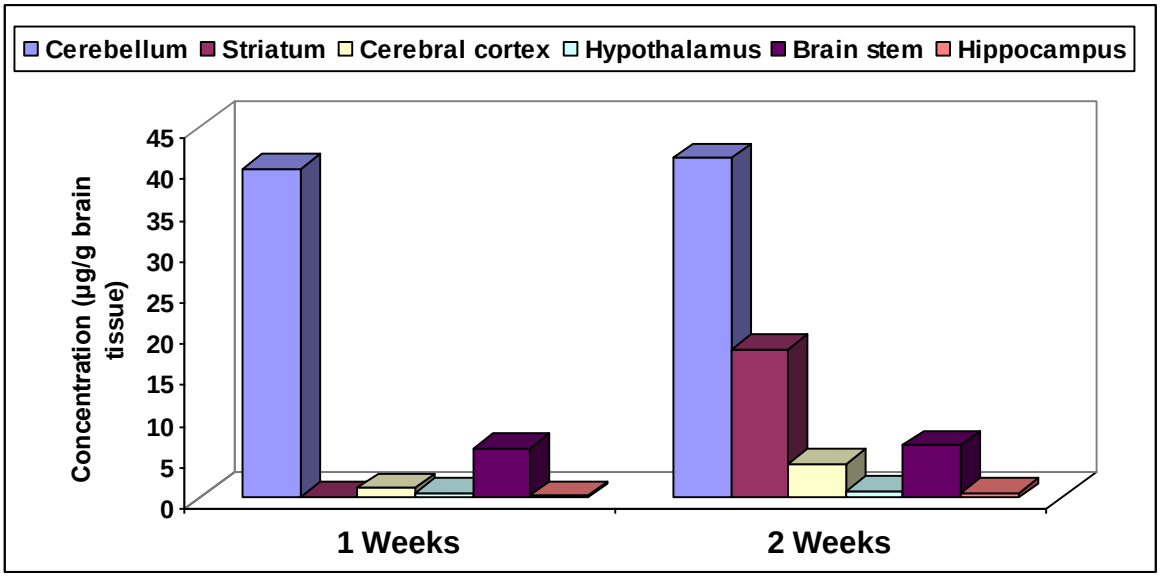

Fig. (10) : Effect of chronic oral administration of pits of data palm and methylprednisolone (20 mg/kg b.wt.)on norepinephrine (NE) content represented by the % difference between control and treated values in the different brain areas of male albino

Table (11): Effect of chronic oral administration the of methylprednisolone (20mg/kg b.wt.), dat palm pits and dat palm pits with methylprednisolone on testosteronel level in serum blood of male albino rat

Time of decapitation		Methylprednisolone (ng/ml) mean ± S.E.	Dat palm pits	Dat palm pits with methylprednisolone
1 week	C	0.817±0.000	0.817±0.000	0.817±0.000
	T	0.630±0.002*	2.588±0.017***	1.381±0.003***
	%	-22.89	216.77	69.03
2 weeks	C	0.818±0.000	0.818±0.000	0.818±0.000
	T	0.640±0.003*	2.605±0.003***	1.410±0.003***
	%	-21.76	218.46	72.37

- Statistical analyses were performed between control (C=6) and treated (T=6) animals by using paired *t*' test
% : Percentage of change from control. *p< 0.05,**p< 0.01 & ***p< 0.001

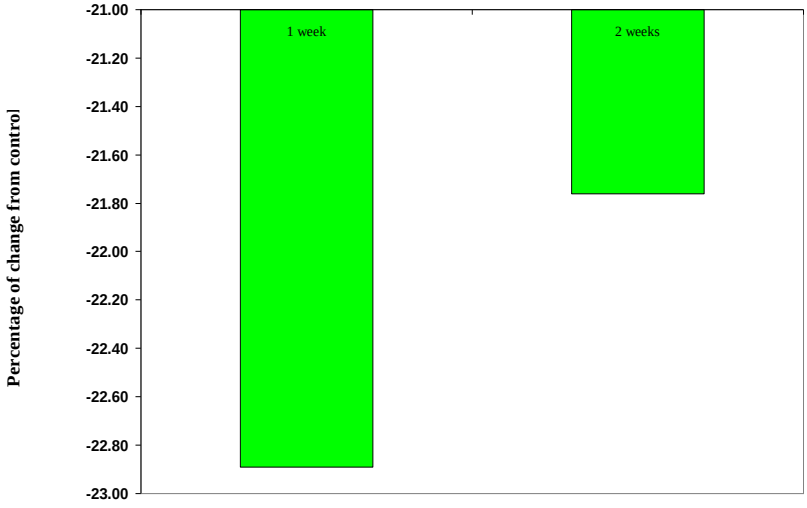

Fig. (11) : Effect of chronic oral administration of methylprednisolone on Testosterone level represented by the % difference between control and treated values in serum of male albino rat

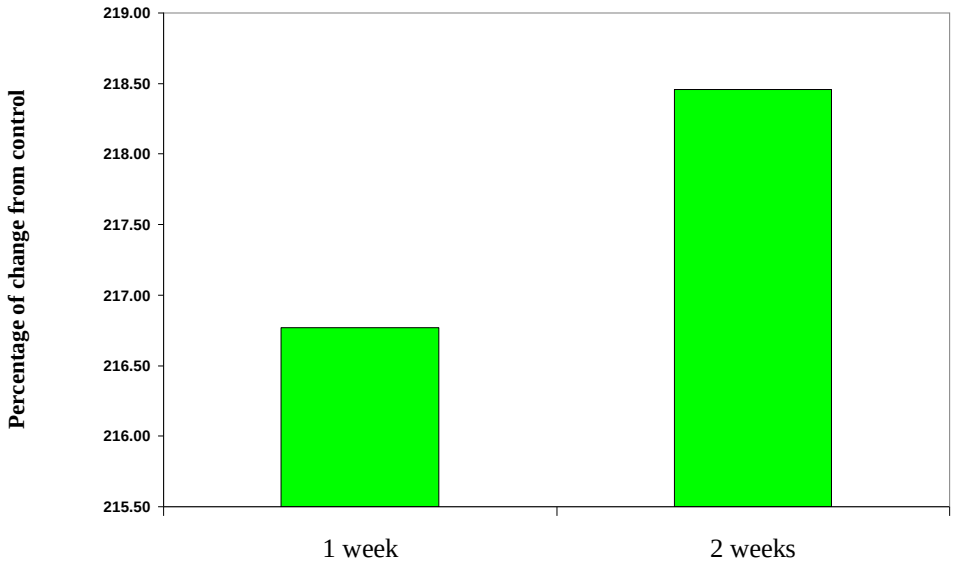

Fig. (12) : Effect of chronic oral administration of pits of data palm on testosterone level represented by the % difference between control and treated values in serum of male albino rat

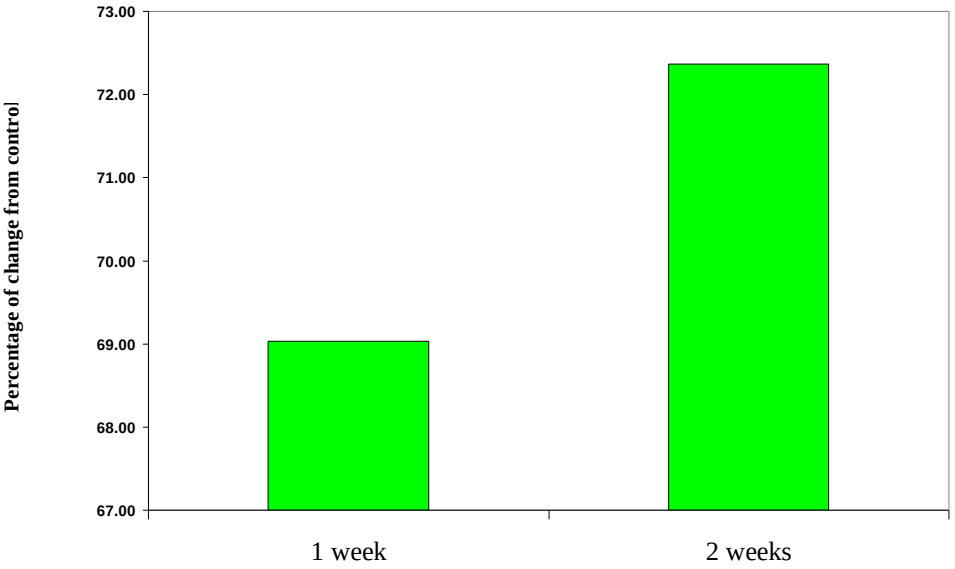

Fig. (13) : Effect of chronic oral administration of data palm pits with methylprednisolone on testosterone level represented by the % difference between control and treated values in serum of male albino rat .

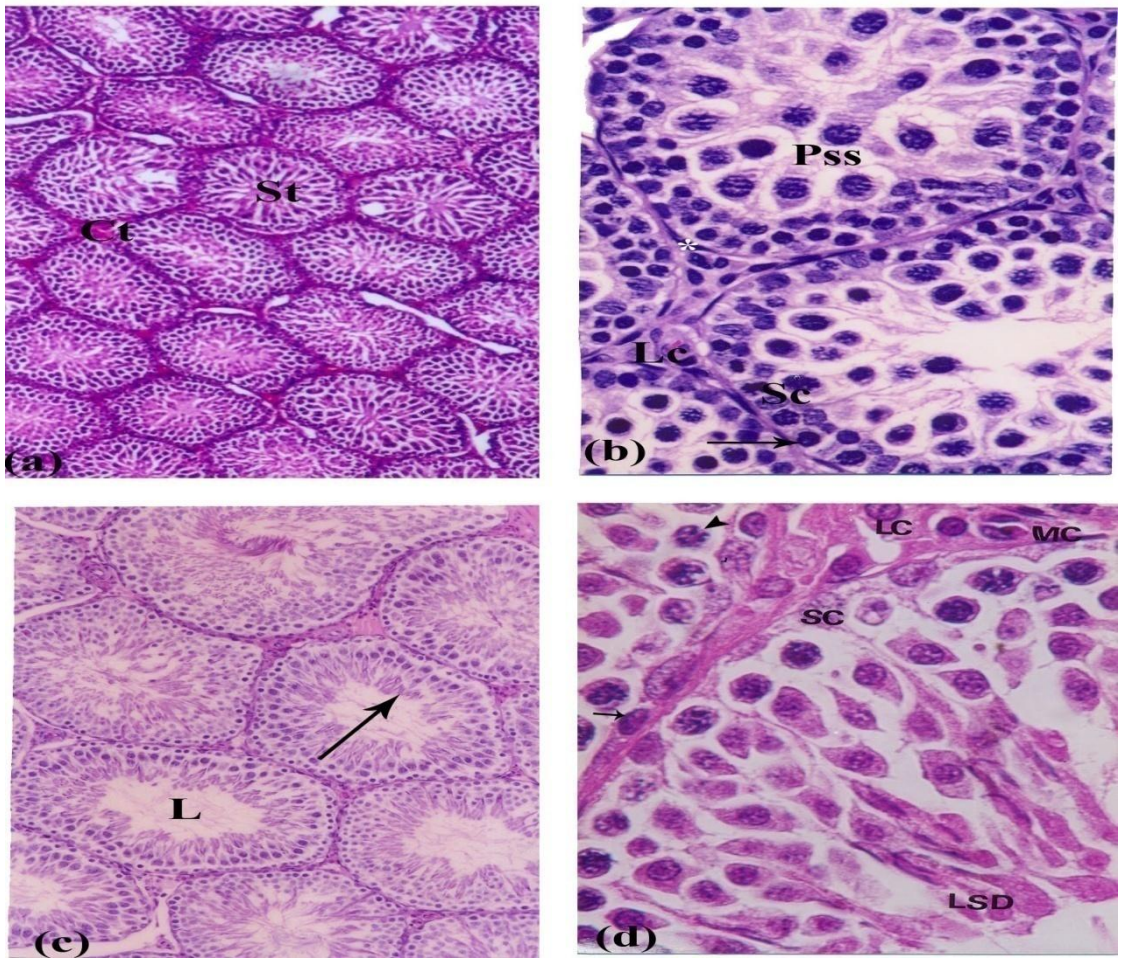

Plate (1a-d): Transverse sections of tests of male rate for control group (G_1). 1a:Note small seminiferous tubules (St) mostly without lumen, surrounded by fibrous connective tissue (CT) layer (H & E; x 100).1b:High magnification showing dark spermatogonium (↗) and pachytene spermatocytes (Pss) and Sertoli cell (SC). Note, myoid cell (*) Layer surrounded St and intertubular space contains leydig cells (Lc) (H & E, x 400).1c: Note, enlarged seminiferous tubules populated by spermatocytes and late spermatids (↗) surround the tubular Lumen (L) (8 weeks of age (H & E; x 100). 1d: High power from 2c showing Light (▲) and dark (↗) spermatogonium adjacent to basal Lamina; late spermatids (LSD) with elongated head directed towards Sertoli cells (Sc). Note, myoid cell (Mc) and Leydig cells (LC)

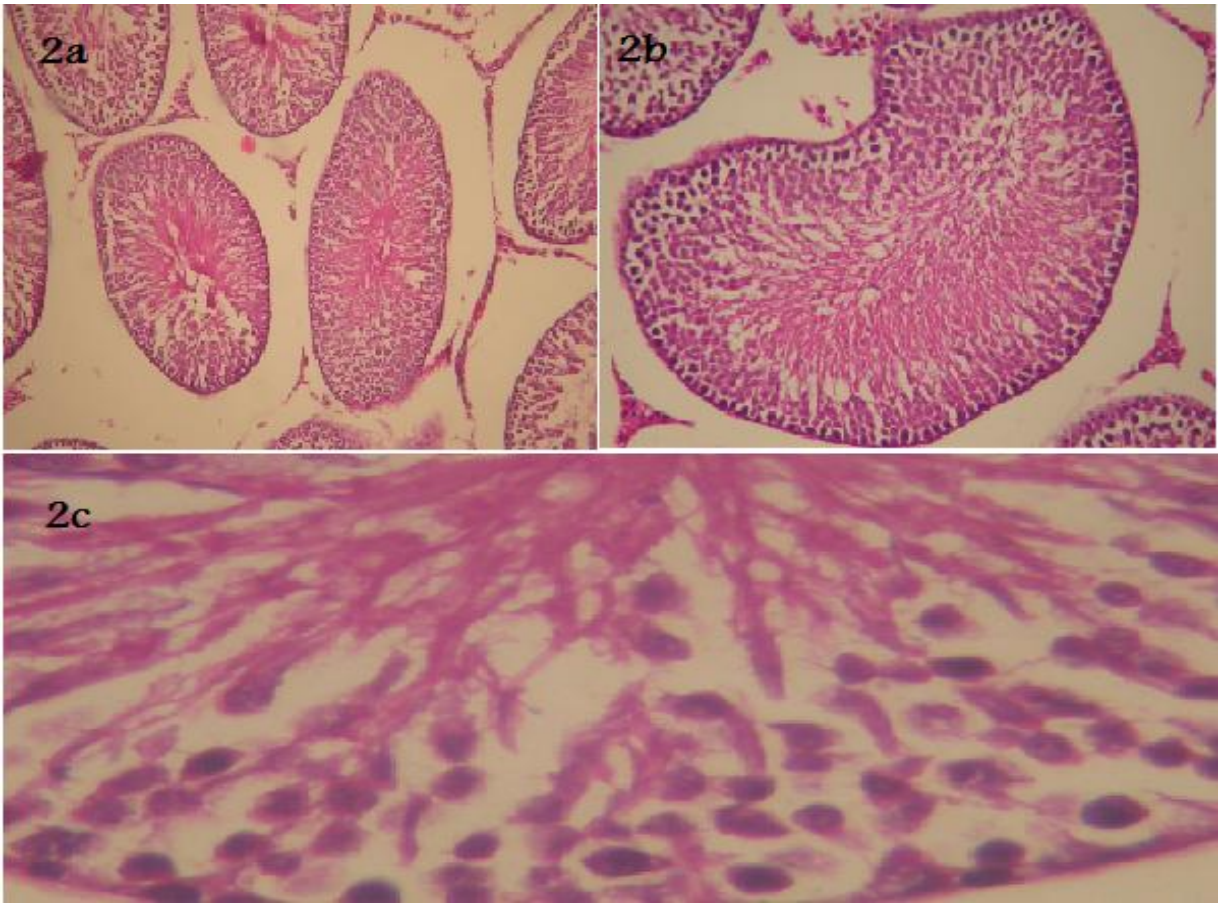

Plate2. Effect of chronic oral administration of pits of data palm on testicular of male albino rat. 2a. A distented tubules showing an increased active spermatogenesis with significant rise of number of mature sperms (H & E; x 40). 2b. tubule showing an increased active spermatogenesis (H & E; x 100). 2c. Marked increase in spermatogenesis, free of early or late arrest. (H & E; x x400)

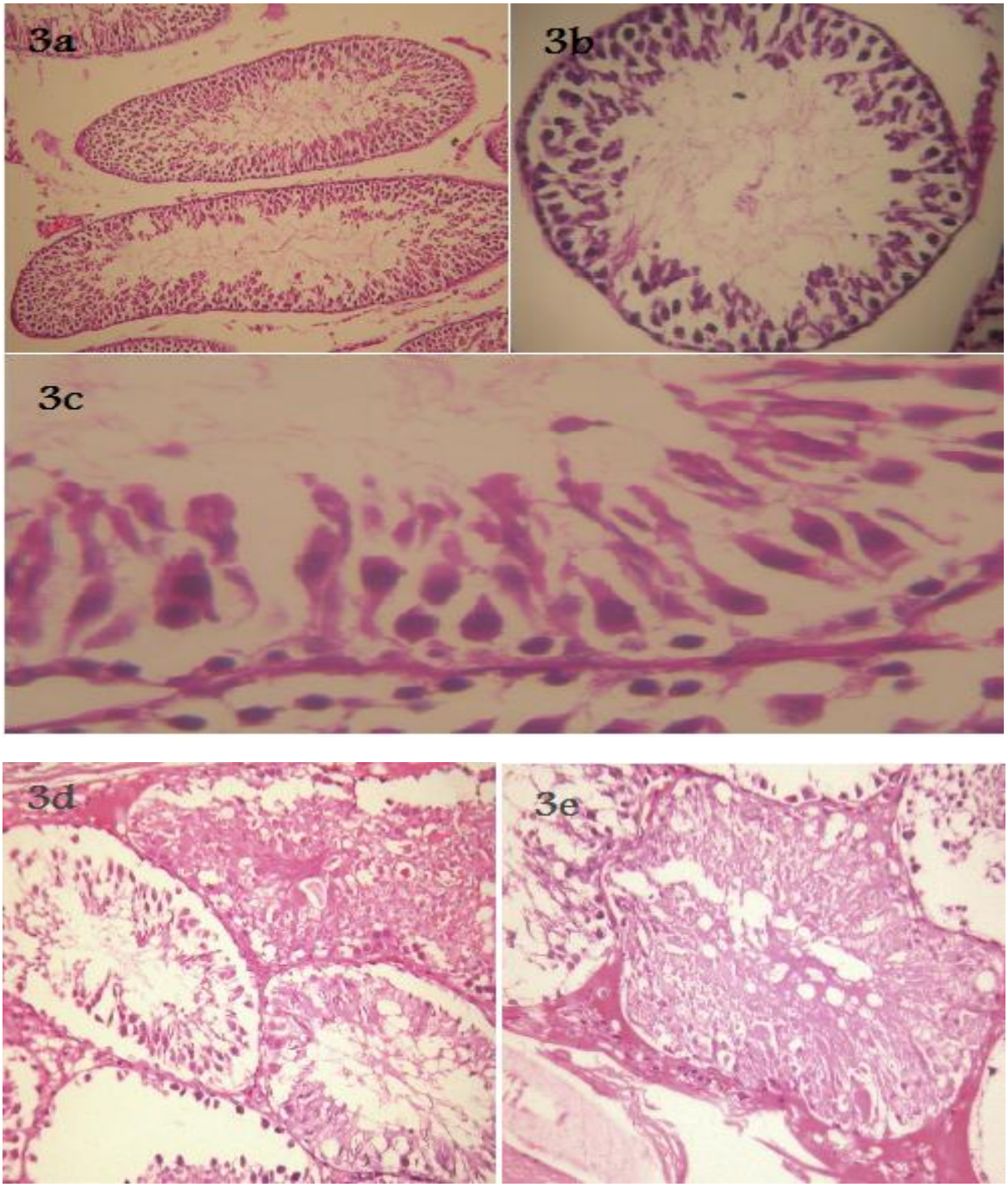

Plate 3. Effect of chronic oral administration of methylprednisolone on testicular of male albino rat. 3a. A distented tubules showing Marked reduction of spermatognesis (hypospermatogenesis) (H & E; x 100) 3 b. tubule showing Partial late arrest with marked reduction of mature sperms (H & E; x 100). 3c. Early arrest with absent mature sperms & germ cell hypoplasia (H & E; x 400. 3d. There are focal areas of disrupted architecture & the tubules show absent spermatogenesis (H & E; x 1000). 4e. Foci of interstitial fibrosis, congestion, vascular injury 'endartritis" & inflammation (H & E; x 400).

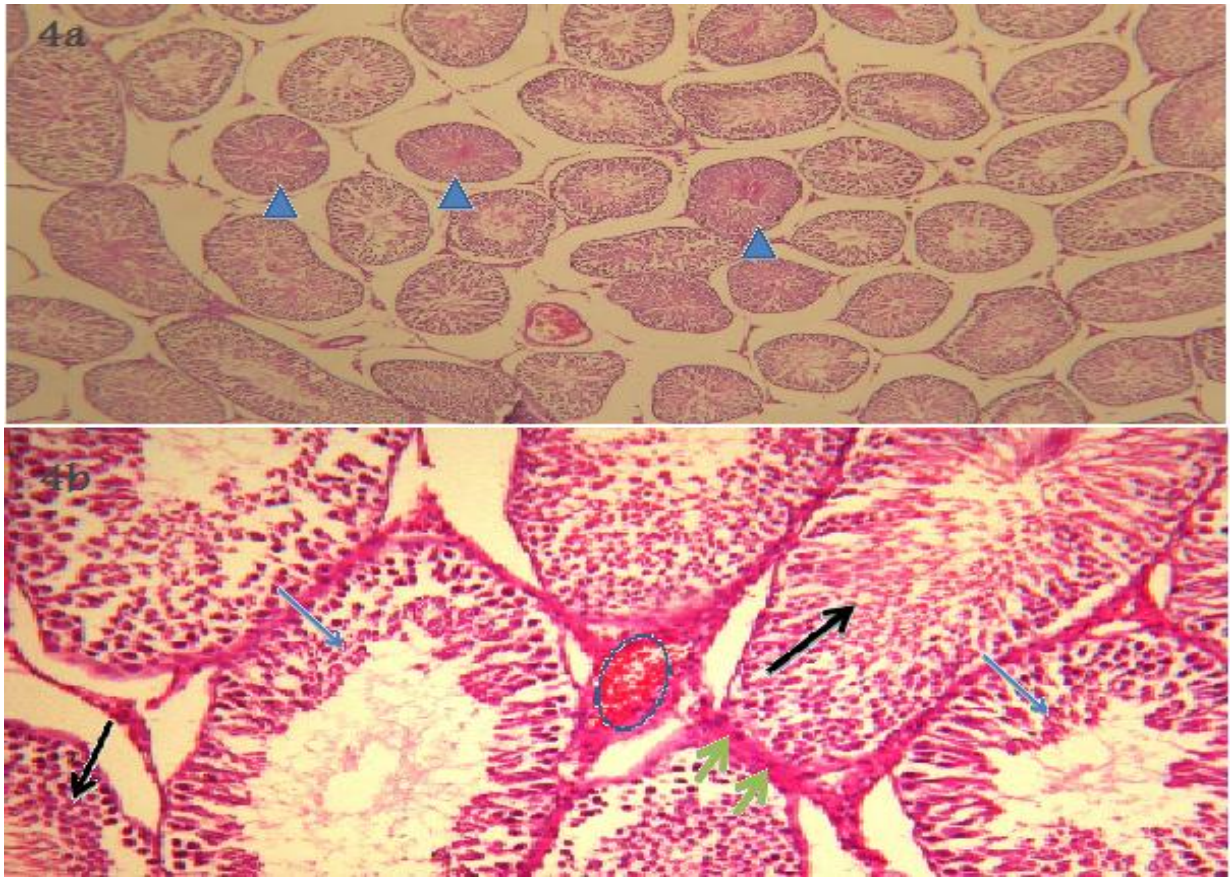

Plate4. Effect of chronic oral administration of methylprednisolone and pits of data palm on testicular of male albino rat. 4a. Recovery effect (H & E; x 40).notice high sperm in some tubules (arrows). 4b. There is partial tissue recovery. Tubular partial late spermatogenic arrest (spermatide level) is only seen in 10-20% of tubules (blue arrows and cycle). Intact tubules varies from hypospermatogenesis to near normal count (black arrows). There is minimal interstitial fibrosis (green arrows).

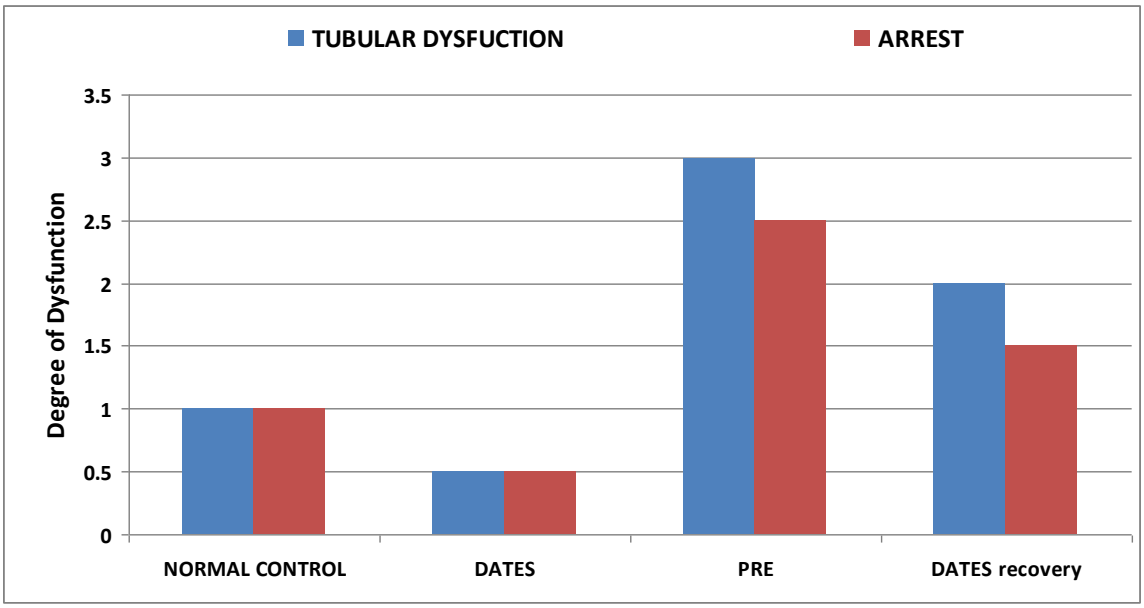

Figure 14. The effect of pits of data palm enhanced spermatogenesis, versus normal control & methylprednisolone (pre). Notice a significant enhanced spermatogensis induced by dates when compared by normal & methylprednisolone effects
Dates recovery= pits of data palm and methylprednisolone, PRE = methylprednisolone Tubular dysfunction and arrest
Dates =

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